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Year XX July & August 1946 Nos. 7 & 8

INTERNATIONAL INSTITUTE OF AGRICULTURE

INTERNATIONAL BULLETIN OF PLANT PROTECTION

PUBLISHED MONTHLY

INTERNATIONAL MYCOLOGICAL INSTITUTE 27 NOV 1946

REPRINT FROM THE 'INTERNATIONAL REVIEW OF AGRICULTURE'

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INTERNATIONAL BULLETIN
OF PLANT PROTECTION

Year XX July & August 1946 Nos. 7 & 8

METABOLISM OF THE WHEAT - *ERYSIPHE GRAMINIS* 'COMPLEX' *

In a previous work [1] I set forth the experimental data obtained on the rate of respiration, glycolysis and transpiration during the full cycle of the disease, for the following 'complexes': haricot bean - *Uromyces appendiculatus*, horse-bean - *Uromyces fabae*, and lettuce - *Bremia lactucae*.

In this report I propose to give the results of similar experiments carried out on the wheat - *Erysiphe graminis* 'complex'. For this disease, I studied, besides the rate of the three aforesaid functions, that of photosynthesis.

Technique employed.

The majority of the experiments were effected on the 'Virgilio' variety of wheat, though the 'Rieti' and 'Mentana' varieties were also used.

For the study of respiration and glycolysis, I adopted the micromanometric method of Warburg (see paper cited). For transpiration, I compared the ratio between fresh and dry weight in the diseased plant, and this same ratio in the healthy plant.

For the study of photosynthesis, however, I developed a simple method of sufficient accuracy and fairly appreciable sensitiveness. It is based, like other similar methods, on the amount of CO_2 fixed by the green tissues [2].

The apparatus used for the study of photosynthesis is composed principally of bell-jars, under which are placed the plants to be observed. These jars have an orifice at the top fitted with a rubber stopper with two openings, one of which remains open to enable the air to penetrate from outside, while the other is connected to two test tubes forming a couple and each containing

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* Communication from Prof. C. Sempio, Plant Pathology Institute of the University of Perugia, Italy.

[1] Sempio, C. Respirazione, glicolisi e traspirazione nel corso del parassitamento. *Ann. della Fac. Agr.*, Perugia, 1942, vol. I.

[2] A similar method was adopted by F. Bruno at Palermo to study the seasonal variations of photosynthesis in evergreen plants ('Studio sulla fotosintesi clorofilliana delle piante sempreverdi nel clima del Mediterraneo'. *Lav. Ist. Bot. di Palermo*, 1936, vol. VII).

50 cc. barium hydrate (approximately N/20) to fix the CO_2 present in the air which has passed through the bell-jars.

Each couple of test tubes is connected to a Mariotte bottle filled with water, serving as suction apparatus. The outflow by drops, and consequently the rate of aspiration and bubbling, are regulated so as to collect, every hour, about 1.5 litres of water, which is measured at the end of the experiment. The quantity of water obtained expresses the volume of air which has passed through each bell-jar and which has bubbled through the test tubes for the entire duration of the experiment, which generally lasts from 4 to 7 hours in full light.

At the end of the experiment, the liquid is left to stand for about 24 hours in order to allow all the barium carbonate formed to deposit ; a certain quantity of the liquid contained in each couple of test tubes is then taken and titrated with N/50 HCl solution. It goes without saying that the solution of barium oxide placed in the test tubes at the beginning of the experiment had been titrated. The difference between the titre of this solution and that of the solutions contained in each couple of test tubes in the experiment gives the quantity of barium hydrate removed from the original solution by the CO_2 contained in the air bubbled. A simple calculation gives the quantity of CO_2 contained in the air which passed through each of the bell-jars.

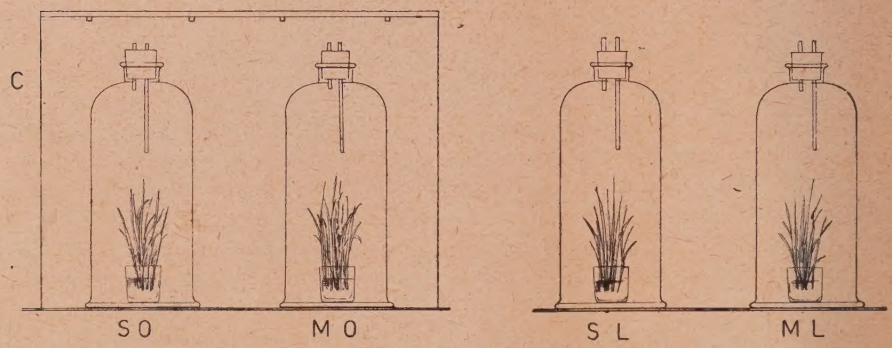


FIG. 1. — SO = healthy plants placed in the dark.
MO = diseased plants placed in the dark.
SI, = healthy plants exposed to the light.
MI, = diseased plants exposed to the light.
C = wooden box, with removable cover, painted black on the inside and light-tight.

However, as the plants exposed to light carry out the functions of respiration and photosynthesis at the same time, it is necessary, if it is desired to ascertain the exact quantity of CO_2 fixed by photosynthesis, to specify the quantity of CO_2 emitted in respiration and which has to be added to the CO_2 contained in the surrounding air.

In this case, for both the diseased and healthy plants (the latter serving as control), two bell-jars are employed : one is kept in the dark to ascertain the exact quantity of CO_2 emitted in the course of respiration, the other is exposed to full light, to enable the plants inside the jar, not only to respire but to accomplish their photosynthesis. The difference between the quantity of CO_2 contained in a certain volume of air which has passed through the bell-jar placed in the dark, and that of the same volume of air which has passed through the bell-jar exposed to the light, gives the quantity of CO_2 fixed by photosynthesis, by the diseased plants or by the healthy plants. The ensuing calculations always refer to 1 gm. of dry matter and to 10 litres of air.

These calculations give the extent of the intensity of the photosynthesis process in both diseased and healthy plants, and consequently enable easy comparisons to be made.

The above method was checked by the method of evolution and measurement of O_2 with *Elodea canadensis*, and gave results which corresponded exactly.

The plants were grown in earth in pots. When the second leaf had attained a sufficient development, the pots were divided into two lots ; those of the first lot served as control (with healthy plants), the others were taken to a small greenhouse where the plants were infected by dusting them with large quantities of conidia of the fungus. Metabolism tests were effected every 2 or 3 days, according to the rapidity of the development of the disease, and up to its maximum stage.

At the time of the photosynthesis test, the plants were cut at the level of the collar, and placed in glasses with a little water to prevent them withering. (This precaution was necessary to avoid errors sometimes due to a difference in the quantities of CO_2 given off through fermentation of the soil contained in the pots, which very likely were to be attributed to a difference in the content of the decomposing organic matter in the various pots).

A clearer idea of the method in question will be obtained from figures 1 and 2.

Discussion of results.

The results of the experiments are assembled in tables I, II and III, which summarize, respectively, the data of the two series of experiments on respiration, photosynthesis and glycolysis. Tables I and III also indicate, in percentage, the ratios fresh weight : dry weight, which served to draw the transpiration curves.

Graphs I, II and III give a synthesis of these tables. Taking the value of the different functions with the healthy plants as equivalent to 100, the curves of the values taken by these same functions with diseased plants were plotted throughout the complete course of the disease. Consequently a clear picture is obtained of the modifications produced in the plant by the disease.

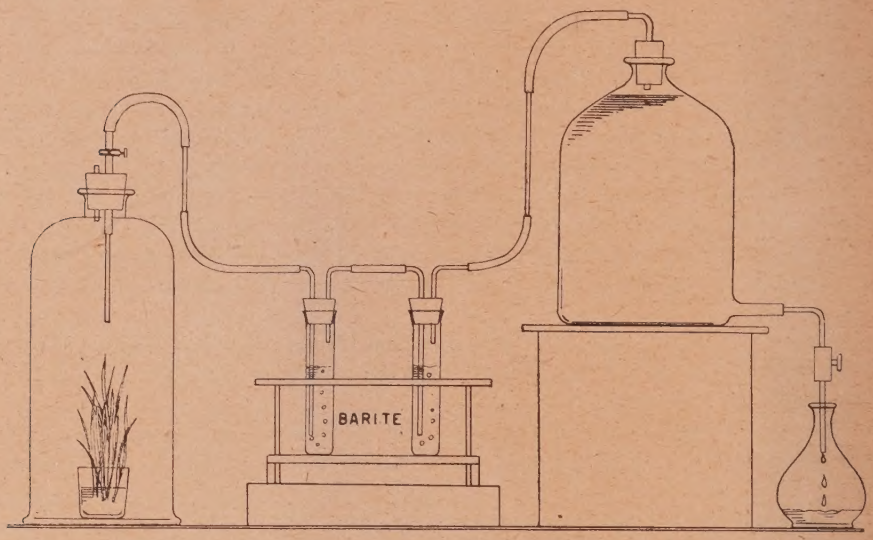


FIG. 2. — Detail of the preceding figure, showing the manner in which each bell-jar is connected to two test tubes and a aspirator bottle. For the sake of clarity, the test tubes and the bell-jar have not been drawn in proportion. Note that the air from the bell-jar is drawn up from close to the leaves, in order to obtain a more exact indication of the CO_2 concentration of the layer of air immediately surrounding the leaves.

It ensues from graphs I and II that, with plants infected with *Oidium moniloides* conidia, the four functions are modified, compared with healthy plants, as follows : —

(1) Respiration begins by being slightly higher than that of the healthy plant ; subsequently it rises rapidly and attains very high values, the maximum occurring at the time of the differentiation of the conidiophores and the conidia : this maximum may be four times the normal value. The intensity of respiration then diminishes a little according as the disease approaches its conclusion, but it always remains well above normal.

(2) Photosynthesis, on the contrary, is definitely stimulated during the first 2 or 3 days following inoculation of the disease, probably when the suctorial organs penetrate into the epidermis cells ; during expansion of the mycelium, it falls to values practically always inferior to the control ; then it rises to values appreciably higher than the normal during the period of differentiation of the conidiophores and the conidia ; finally it again drops, more or less rapidly, to values much lower than normal to attain, in the advanced stage of the disease, values below half those noted for the healthy plant.

(3) Glycolysis is appreciably stimulated in the early days of incubation ; it then descends below normal, and this difference (with the normal), in general, increases progressively as the disease draws to an end.

TABLE I. — Respiration experiments carried out on 'Virgilio' wheat infected with *Oidium moniloides* conidia.

Number of experiments	Number of days elapsed since onset of infection	Ratio between fresh weight and dry weight in % Average value		Q_{O_2}		Average Q_{O_2} Values for each experiment		Q_{O_2} values in infected plants, the corresponding values in the healthy plants being = 100	Temperature of bath during experiment C	OBSERVATIONS
		Healthy plants	Infected plants	Healthy plants	Infected plants	Healthy plants	Infected plants			
Ist Series										
1	2	9	9.2	1.76 1.70	2.14 1.97	1.73	2.06	**119.1**	24.6°	*Experiments on 1st leaf*
2	4	9.7	9.5	0.92 0.87	1.18 1.17	0 90	1.18	**131.1**	23.1°	Large numbers of suckers
3	6	8.7	9.6	1.18 1.11	2.07 2.01	1.15	2.04	**178.2**	23.5°	Mycelium evident
4	8	8.7	9.3	1.20 1.20	3.13 3.67	1.20	3.40	**283.3**	24.5°	Beginning of formation of conidiophores and conidia
5	11	8.7	10.5	0,82 0.90	3.52 3.94	0.86	3.73	**433.7**	24.6°	Conidia abundant; very severe attack
6	14	9	10.2	0.81 0.81	2.91 3 28	0.81	3.09	**382.1**	23.8°	Conidia very abundant; idem
IInd Series										
1	1	11.9	11.3	1.77 1.54	2.08 2.05	1.65	2.07	**124.8**	24.9°	*Experiments on 2nd leaf*
2	3	10.9	11.5	1.63 1.59	3.08 2.86	1.61	2.97	**184.6**	25.3°	Mycelium developed, with numerous suckers
3	5	9.5	11.2	1.24 1.23	3.96 3.92	1.23	3.94	**319.7**	23.8°	Formation of conidiophores and conidia
4	7	9.8	10.5	1.28 1.30	3.88 4.11	1.29	4.00	**309.7**	24.4°	Conidia very abundant
5	10	10.7	11 —	1.13 1.36	3.74 3.50	1.25	3.62	**290** —	24.4°	Very heavy attack
6	12	10 —	11.8	1.26 1.19	3.09 3.28	1.22	3.18	**260** —	24.4°	Very severe attack; leaves more or less withered

TABLE II. — Photosynthesis experiments carried out on wheat ('Virgilio' and 'Rieti' varieties) infected with *Oidium moniloides* conidia.

Number of experiments	Duration in hours	Number of days elapsed since onset of infection	Weather	Temperat. in C	Mg. CO_2 fixed by 1 gm. dry matter in 10 litres air		Quantity CO_2 fixed by infected plants taking as 100 that of CO_2 fixed by healthy plants	OBSERVATIONS
					Healthy plants	Infected plants		
Ist Series								
1	6.30	2	bright	16°–22°	24,07	41,26	**171.2**	*Experiments carried out on 1st leaf of 'Virgilio' variety*
2 —	6.30	4	cloudy	15°–16°	20.65	24.30	**117.7**	Mycelium starts to develop; suckers
3 —	7.30	6	bright	13°–17°	27.05	18.07	**66.7**	Mycelium well developed
4 —	8 —	8	bright	14°–20°	19.30	26.76	**138.7**	Conidiophores and conidia formed
5 —	6.30	11	half overcast	16°–20°	17.88	22.03	**123.2**	Conidia very abundant; very severe attack
6 —	4.15	14	fine	16°–23°	20.07	9.19	**45.7**	Conidia very abundant
IInd Series								
1	6.30	1	bright	21°–27°	5.96	8.88	**149** —	*Experiments effected on 1st and 2nd leaves of 'Virgilio' variety*
2 —	7 —	3	bright	19°–29°	8.47	7.94	**93.7**	Suckers; mycelium developed
3 —	7 —	5	overcast	17°–20°	7.08	13.76	**194.4**	Start of formation of conidiophores and conidia
4 —	7.15	7	nearly cloudless	14°–26°	7.56	11.10	**146.8**	Conidia abundant; heavy attack
5 —	6.15	10	overcast	17°–21°	30.90	29.30	**94.8**	Conidia very abundant
6 —	7.45	12	nearly cloudless	12°–22°	22.55	3.22	**14.3**	Very severe attack; plants somewhat withered
IIIrd Series								
1	3	2	very bright	22–28°	5.23	15.36	**293.7**	*Experiments carried out on 1st leaf of 'Rieti' variety*
2	3	4	very bright	24–29°	4.58	4.60	**100.4**	Development of mycelium
3	3	7	very bright	24–29°	4.83	7.57	**156.7**	Formation of conidiophores and conidia
4	4	11	very bright	26–32°	6.39	1.48	**23.2**	Conidia abundant; severe attack

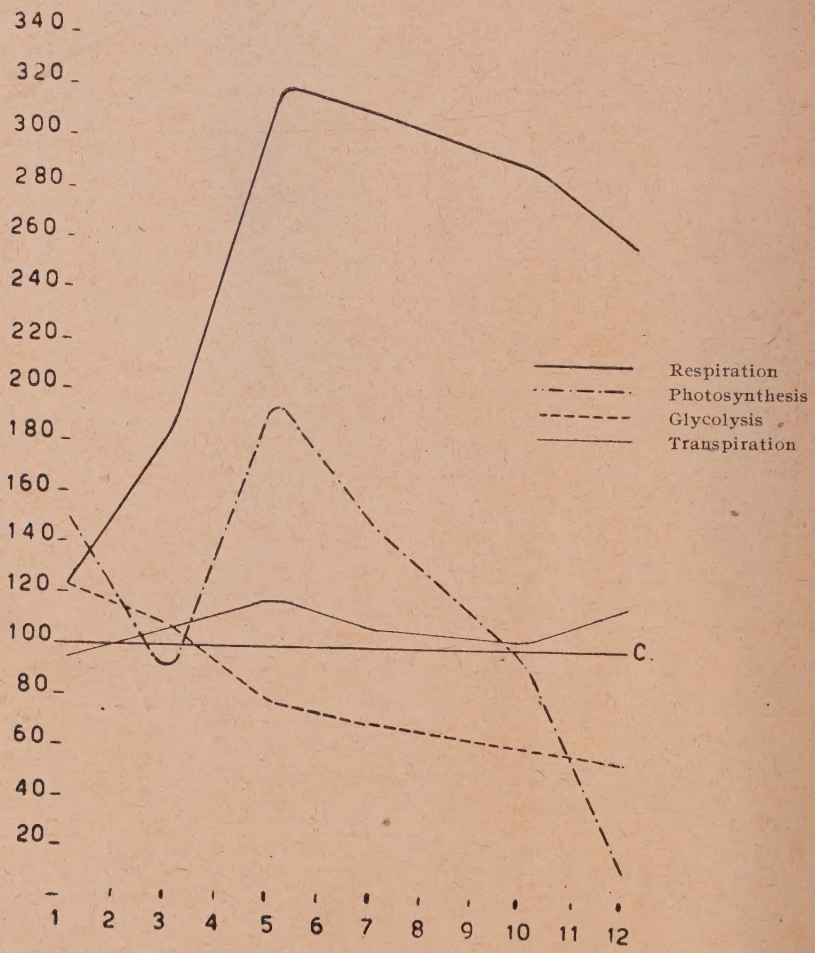
NOTA. — In all the experiments of the 1st series and in the last two of the 2nd series, the air in the bell-jars was enriched with small doses of CO_2, the same amount for each jar. In effect, there is found to be a much more intensive fixation of CO_2 in these experiments than in the others, because the CO_2 concentration is closer to the optimum.

In the experiments with the first leaf, the second leaf was cut before infection; subsequently as it grew it was always cut before each experiment. This was also done with the third leaf in the experiments on the first and second leaves, in order not to include in the experiment leaves or parts of leaves not infected.

TABLE III. — Glycolysis experiments on wheat ('Virgilio' and 'Mentana' varieties) infected with *Oidium monilioides* conidia.

Number of experiments	Number days elapsed since onset of infection	Ratio between fresh weight and dry weight in % Average value		$Q^N_{CO_2}$		Average of $Q^N_{CO_2}$ values for each experiment		$Q^N_{CO_2}$ values in infected plants, the corresponding values in the healthy plants being = 100	Temperature of bath during experiment C.	OBSERVATIONS
		Healthy plants	Infected plants	Healthy plants	Infected plants	Healthy plants	Infected plants			
Ist Series										
1 —	2 —	11.7	11.2	0.78 1.12	1.26 1.08	0.95	1.17	**123 —**	24.5°	*Experiments on 2nd leaf; 'Virgilio' variety*
2 —	4 —	10.6	10.8	1.05 1.09	1.20 1.12	1.07	1.16	**108 7**	24.5°	Suckers
3 —	6	11.8	11.7	1.12 0.99	0.83 0.84	1.05	0.83	**79.3**	24.4°	Mycelium evident
4 —	8	10.7	12.3	0.86 0.85	0.55 0.65	0.86	0.60	**70 6**	24.4°	Formation of conidiophores and conidia
5 —	11	11.1	11.4	1.07 1.01	0.65 0.64	1.04	0.65	**62.1**	24.5°	Very heavy attack
6 —	14	11.4	13.5	0.95 0.65	0.45 0.45	0.80	0.45	**55.9**	24.4°	Very severe attack; withering of leaves
IInd Series										
1	1	8.93	9.38	0.58 0.73	0.79 0.84	0.66	0.82	**123.9**	25.3°	
2 —	2 —	9.03	9.12	0.79 0.58	0.72 0.75	0.68	0.73	**107.5**	24.8°	*Experiments on 1st leaf; 'Mentana' variety*
3 —	4 —	8.33	9.23	0.76 0.73	0.72 0.72	0.74	0.72	**95.9**	25°	Suckers abundant
4 —	6 —	8.48	8.90	0.62 0.57	0.68 0.58	0.60	0.63	**106.6**	25.3°	Mycelium well developed
5 —	8 —	8.50	9.19	0.74 0.74	0.65 0.63	0.74	0.64	**87 —**	25.3°	Formation of conidiophores and conidia
6 —	11 —	8.70	9.93	0.84 0.86	0.52 0.64	0.85	0.58	**68.2**	25.1°	Conidia abundant; attack considerable

(4) At first the traspiration has practically the same intensity as in healthy plants; it subsequently increases, towards the 3rd or 4th day, to values slightly higher than the normal, where it remains until the end of infection.



GRAPH I. — *Abscissae*: number of days elapsed since the onset of infection. The deeper stressed marks indicate the days on which the determinations were made.

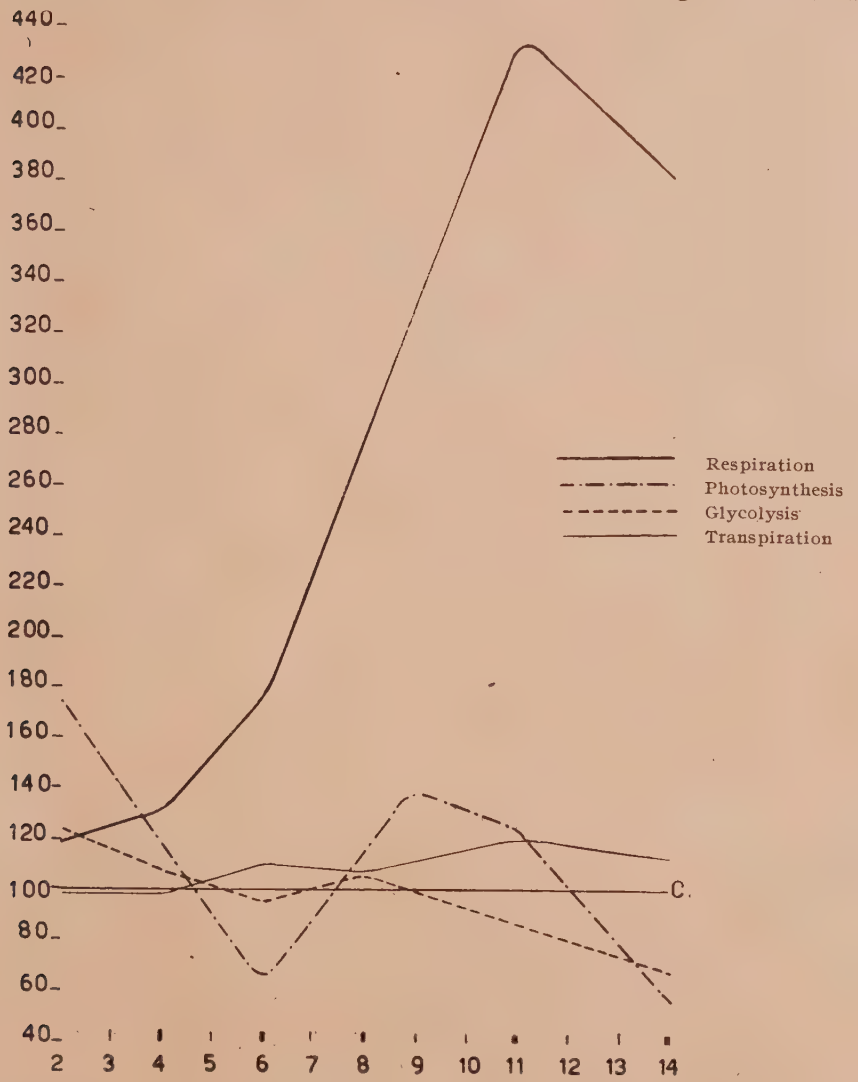
Ordinates: Values of the different functions with the diseased plants, compared with the values verified on the healthy plants. C = 100.

These data also apply to the other graphs.

* * *

Let us now attempt to interprete these experimental data in the light of other experiments I reported in a paper which dates back to 1938 [3].

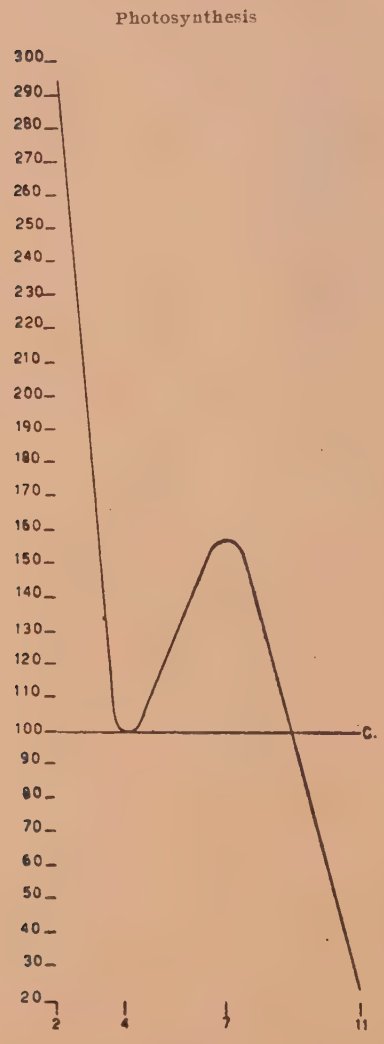
I then remarked that with certain diseases, including wheat *Oidium*,



GRAPH II (see key to graph I).

[3] SEMPIO, C. Influenza della luce e dell'oscurità sui principali periodi del parassitamento. *Riv. di Pat. veg.*, 1939, vol. XXIX.

if the infected plants are left in the dark during the first period of parasitism, namely, during the first 3 or 4 days incubation, there was always a more or less marked excitation of the disease, compared with infected plants exposed to normal light. On the other hand, I observed that if diseased plants are subjected to this treatment (kept in the dark) during the third period of parasitism, that is to say from the 6th or 7th day to the 10th day, the opposite effect was obtained: the intensity of attack is always more or less definitely attenuated compared with plants which have not been subjected to this treatment. I also called attention to the fact, of a certain interest in itself, that the same treatment (under the circumstances, keeping the plants in the dark) may have diametrically opposite effects on the progress of the disease, according to whether it is applied during the first or the third period of parasitism.



GRAPH III.
(see key to graph I)

Now, the experiments which I am reporting here explain and supplement these remarks; nay more, the two series of experiments each make the other clear. In comparing side by side the results of these two series of experiments, it seems that it might be concluded that the initial stimulation of photosynthesis which is noted in infected plants should be interpreted as a defensive reaction of the plant against the action of the parasite tending to penetrate it. This is so true that, if the plant is prevented from reacting by this natural metabolic defence (by blocking photosynthesis during the first period of parasitism), attack is seen to be definitely more intense than on plants which at the beginning were able to benefit not only from normal photosynthetic intensity, but particularly from this reaction through stimulation. It is to be noted that this type of metabolic defence, although only of ephemeral duration, is, however, not to be disregarded, as it can check appreciably and even definitely the installation of the parasite in the host plant. This stimulation can, in fact, be even

very marked, as is shown by graph III, plotted from the data of the third series of experiments assembled in table II.

On the other hand, if the infected plants are subjected to the inverse treatment, namely, if they are exposed to continuous light (artificial) during the first four days of parasitism [4], there is seen to be a decided diminution in intensity of attack compared with plants which were allowed nocturnal repose. It is evident, therefore, that if the plant is allowed to continue its photosynthetic defence, the efficacy of this defence is distinctly augmented.

The same applies to glycolytic activity. This function in effect also undergoes, as a result of parasitic attack, an appreciable stimulation, compared with the normal state, during the first two or three days of incubation. On the other hand, other experiments [5] showed that if the plants are left in the dark, not only is photosynthesis definitely stopped, but the glycolytic capacity of the tissues is considerably influenced, to the extent of falling below half the normal.

In the papers cited (4, 5) attention has been called to the significant connection existing, especially in the case of *Oidium* on wheat, between the intensity of the glycolytic capacity and the self-defence of the plant, to the extent that this capacity can be defined as if not the cause, at least as an *indication* of the metabolic capacities of resistance of the wheat to *Oidium*. Now, the results of the experiments we have explained also confirm that the increase in glycolytic activity at the beginning of infection, likewise the stimulation of photosynthesis, should be interpreted as basic lines of reaction opposed by the host plant to the attack of the parasite. In effect, when the plant is placed in the dark, not only is the glycolytic capacity of the tissues hindered, but its intensity is reduced to approximately half the normal.

On the other hand, as we emphasized in a previous work [4], if the infected seedlings are exposed to continuous light, a very strong resistance to *Oidium* is obtained, and in a parallel way an increase in the glycolytic capacity in the tissues of the host plant.

In brief, it seems evident, therefore, that the stimulation of photosynthetic and glycolytic activities, characterizing the first period of parasitism, should be considered as an effective form, but unknown up to the present, under which the self-defence of the host plant is manifested, and that this reaction of defence should be entered in the completely vital plan of metabolic resistance.

* * *

We have seen, in studying the progress of the photosynthesis process in the diseased plant, that the intensity of this function again rises markedly

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[4] SEMPIO, C. Influenza della luce continua sulla resistenza del frumento all'*Erysiphe graminis*, e meccanismo di tale resistenza. *Riv. di Biol.*, 1943, vol. XXXV.

[5] SEMPIO, C. Influenza dell'oscurità sulla respirazione e sulla glicolisi di alcune piante in rapporto alla loro resistenza a parassiti obbligati. *Riv. di Biol.*, 1943, vol. XXXV.

above normal when the differentiation of conidiophores and conidia occurs, namely, during the third period of parasitism.

On the other hand, we have noted that, if the infected plants are left in the dark during this period, there is a decided diminution in the intensity of the final attack. This clearly shows that this second maximum of photosynthetic activity should not be considered as a defence reaction of the host plant, since, when photosynthesis is checked during the third period of parasitism (that is to say at the moment when the second maximum of photosynthetis takes place), not only does the parasitic attack not augment in intensity, but, on the contrary, it undergoes a very marked drop.

We think that this excitation should be interpreted rather as a passive reponse of the plant, by now dominated by the parasite, to the stimuli connected with the food requirements of the pathogenic germ. In other terms, the host plant, which appears to have lost entirely the control of its defence, is probably led to aid passively the parasite in meeting its special alimentary requirements, which characterize the conidial differentiation phase [6]. Conse-

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[6] It should be recalled in this respect that during the third period of parasitism, as well as in the subsequent periods, the respiration of the host-parasite *complex* is very intense. Also the air which circulates in the infected tissues is, during this period, much richer in CO_2 than that which circulates in the healthy tissues. This point is worth noting as it would appear from the experimental results available to date that the larger quantity of CO_2 contained in the diseased tissues approximates, to a much greater extent than that contained in the healthy tissues, the optimum concentration for the most intense photosynthetic activity.

Also, as long as the diseased tissues maintain a certain vitality, a more intense fixation of carbon is noted. It may even be taken, without fear of deviating too much from the truth, that, during this period, the reciprocal internal exchange (between the tissues of the plant) is particularly intense, as each maintains its activity on the waste of the other (O_2 and CO_2 respectively). This is why fairly frequently during experiments there is seen to be a persistence of a stimulation of photosynthesis (which cannot otherwise be explained), even in plants which have already yellowed appreciably and which are approaching the final stage of the disease. In these cases, however, photosynthetic activity drops suddenly, while the respiration rhythm continues to remain much higher than normal.

Consequently, an attempt was made in some experiments to enrich the air in the bell-jars with small (identical) quantities of CO_2 so as to exceed, for both the healthy and the diseased plants, the optimum concentration in order that photosynthesis could take place under the best conditions. It was found that, with a slight excess of CO_2, the healthy tissues always definitely proved more capable of fixing the carbon than those in which infection was in an advanced stage (that is to say, towards the end of the third period and in the subsequent periods). It appears evident, therefore, that the second photosynthetic stimulation is due, in the cases where it is prolonged beyond the third period, much more to an environmental cause (in this case, to the higher CO_2 content of the infected tissues) than to a cause which could be called intrinsic, namely, in relation with the stimulus provoked by the parasite on the enzymatic mechanism of its host.

Moreover, what we have just stated in this note is consistent with what is known on the marked positive influence which, within certain well defined limits, a higher CO_2 concentration has on the intensity of the photosynthetic process.

quently, in checking (by means of darkness) the photosynthesis of the plant during this period, which is decisive for the subsequent development of the parasite, it is possible to maintain within moderate limits the final burst of the disease.

In the course of the photosynthetic process of the wheat-*Erysiphe* ' complex ', therefore, two successive periods of stimulation have to be distinguished : the first, shorter but generally more intense, when the parasite sinks in its suctorial organs, and the second, usually less intense but longer, at the time of the differentiation of the conidiophores and the conidia. Between these two maxima, there is a short period of depression in photosynthesis, at the time of the expansion phase of the mycelium. It would seem, however, that these two stimulations ought to be attributed to diametrically opposite causes, in the functional framework of the wheat-*Erysiphe* ' complex '. The first, in fact, has all the characteristics of an effective defensive reaction, while the second (which partly benefits from the higher CO_2 concentration of the air surrounding the infected tissues) appears rather as a functional disequilibrium of the plant, which has become incapable of directing and controlling its functions, disequilibrium which is produced solely for the benefit of the pathogenic microbe which caused it.

This is why, when plants are kept in the dark during the first period of the attack, an important defensive weapon of the host plant is enfeebled, and later it is found that the attack is more severe at the end of the infection cycle. On the other hand, if plants are submitted to the same treatment during the third period of parasitic action, the pathogenic microbe is prevented from exploiting for its benefit the loss of control of its host, and it is subsequently found, at the end of the cycle, that the attack is markedly less intense.

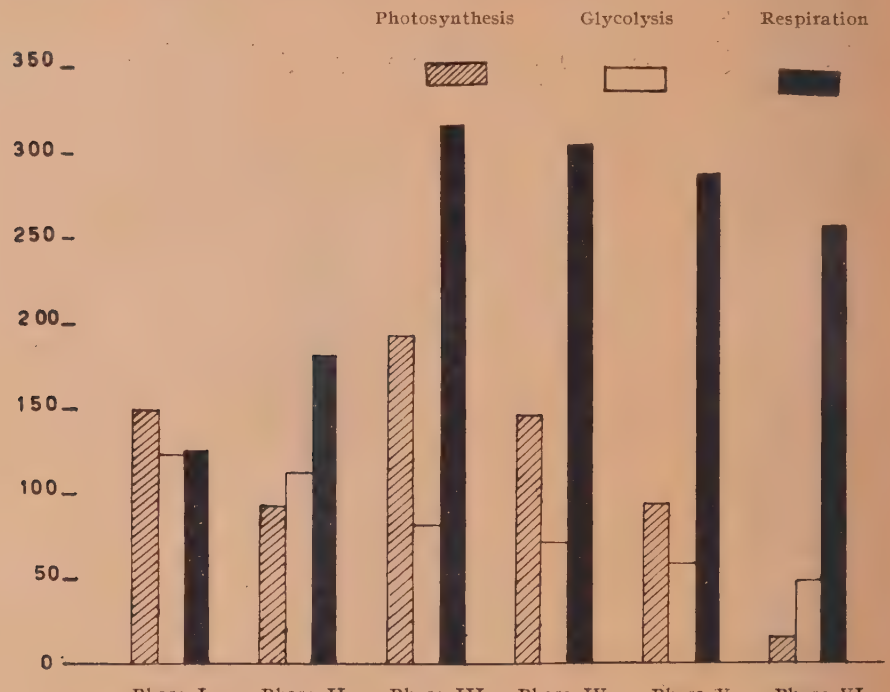
* * *

As regards the essential mechanism according to which the two excitations of photosynthetic capacity check or stimulate the development of the parasite, evidently nothing definite can be stated until a thorough knowledge is obtained of the working of the different enzymatic activities which control the execution of these phenomena or which are closely connected with them. We intend to undertake, in collaboration with experts, difficult and complex experiments of a bio-chemical nature, on this subject.

We can already, however, independently of the success of these experiments and the results which will be obtained, make some general statements which we consider well founded.

In the case under study, it seems that we have a deviation of the nutrient system of the host-parasite complex, and in particular, a displacement of the equilibrium between soluble products, which the parasite can rapidly assimilate, and insoluble products which it cannot assimilate.

Now, it is probable that when the synthesis processes prevail, the tissues of the host are richer in compounds unassimilable or not easily assimilable by the parasite, which consequently finds itself in an environment



GRAPH IV (see key to graph I)

unfavourable for its development. On the other hand, when the deterioration processes (directly or indirectly connected with the rhythm of respiration) prevail, the tissues of the plant ought evidently to be very rich in soluble compounds which the parasite can assimilate rapidly.

In this respect, let us consider graph IV which was plotted from the same series of experiments as graph I. It shows clearly that the first phase of the disease (sinking in of the suctorial organs) is characterized by a general stimulation of the three functions studied, but chiefly of the synthesis processes (photosynthesis) rather than of the deterioration processes (respiration and glycolysis). This predominance appears still more marked in the other experiments (see graph III) than in the case indicated by graph IV. In the second phase (spread of the mycelium) in general, as has been seen, the defence reaction has ended. Nay more, it is during this phase that the plant seems to begin losing the control of its functions, this loss of control becoming more marked as the disease progresses. On the other hand, it is seen that that the processes of synthesis lose their predominance, while those of degeneration become more pronounced ; in effect, the rhythm of respiration is accelerated. In the third and fourth phases (start and completion of the differentiation of conidiophores and conidia) the second stimulation of photosynthesis takes place, but, despite it, the relation between the synthesis

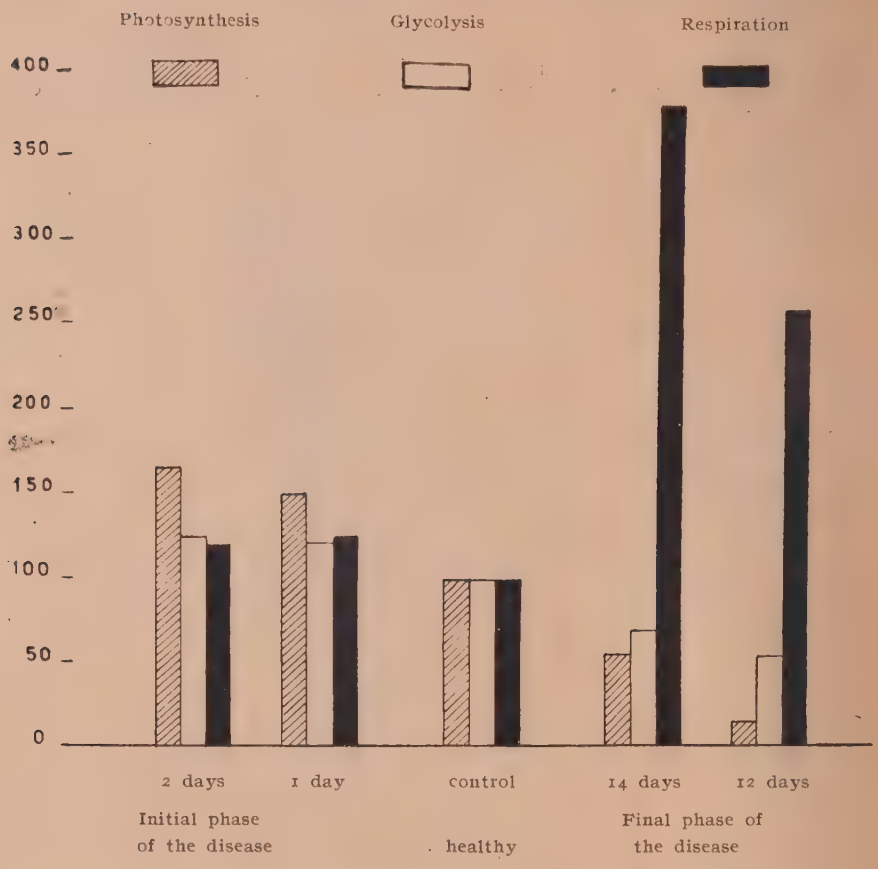
processes and the degeneration processes changes definitely in favour of the latter; this second period of photosynthetic excitation corresponds to the strongest intensity of respiration registered during the full cycle of the parasitic attack [7]. During the fifth and sixth phases, which are the final phases of the disease in which the host is definitely jeopardized, the synthesis processes gradually disappear, while the degeneration processes, connected with the respiration rhythm, remain very active. It is this very marked disequilibrium between the synthesis and the degeneration processes which characterizes the final phase of the disease.

This is quite clear if we turn to graph V, in which the initial and final phases of the two series of experiments represented in graphs I and II are compared.

First of all we note the enormous disequilibrium existing at the end of the disease between the intensity of respiration which remains very high (as much as three times the normal) and the intensity of the photosynthetic and glycolytic capacity which, on the contrary, falls to very low values (even below half the normal value). The ratio between these three basic functions appears greatly altered. Acknowledging, in effect, that, with a normal plant, these three functions have a ratio more or less of 1 : 1 : 1, it is found that with a badly diseased plant, this ratio becomes 3 : 0.5 : 0.5. The ratio between respiration and photosynthesis is even more strongly influenced, and values may stand at 2.5 : 0.5, that is to say, 10 : 1. Such an enormous disequilibrium between these two extremely important functions gives a clear idea of the serious changes caused by the parasite in the normal order of the metabolism of the plant.

This finding raises questions of a more general interest. When it is a question of diseases purposely caused by parasites — in which necessarily a compatibility has to be established enabling an association between the host and its parasite — can it be said that the essence of the disease should be sought for in an impoverishment of the nutrient supply and in the attendant intoxication which the parasite causes by its elimination in the tissues of its host? Or rather should this not be sought for in the *serious functional disequilibrium* which arises in the tissues of the host between the synthesis and the degeneration processes? In other words, the results discussed in this study appear to justify those who seek, even in the case of

[7] When we speak of the process of degeneration we naturally think mainly of respiration, and consider glycolysis as being of minor importance. This is not only because of the great difference in intensity of these two functions, but chiefly because, from the standpoint of the mechanisms of defence linked with metabolism, these two functions have had up to the present, at least for *Oidium* of wheat, an opposite trend. In effect, a strong respiratory intensity is generally a symptom of weak resistance and lack of control in regard to defence, while a high glycolytic capacity usually corresponds to a strong capacity for resistance. (Perhaps because glycolysis causes, as is thought, in the plants an accumulation of ethyl alcohol which might have a toxic action on the parasite).



GRAPH V (see key to graph I).

parasitic diseases, the essence of the disease in the marked functional disequilibriums, that is to say, in the marked changes in the ratios of the principal functions, due, it may be admitted, to an impoverishment in the nutrient supply and to the intoxication phenomena caused in their turn by the parasite.

Lastly, graph V shows the functional physiognomy of the plant in the early stages of the disease, namely, at the moment when it mobilizes its metabolic energies for defence. It may be noted that the principal characteristic of this physiological table is a moderate and balanced stimulation of the chief functions of the plant and particularly, in a very pronounced manner, of photosynthesis.

The contrast between the physiological tables of the first and the second phases of the disease, therefore, is very marked and significant: in the first predominates the stimulation of photosynthesis and glycolysis, which

are both closely linked, directly or indirectly, to metabolic defence ; in the second, on the contrary, it is respiration which predominates, while the other two functions have diminished appreciably compared with normal.

This intense respiratory activity, particularly when it is not balanced by a corresponding stimulation of the two other functions, constitutes, therefore, the dominant expression of the enormous functional disequilibrium characteristic of the stage of the disease, and certainly contributes towards establishing, to a large extent, the nutrient medium favourable to the development of the parasite. It would seem that for this reason it should be considered, at least in the case just studied, as a sign of the more or less complete *collapse* of the defensive powers of the plant.

FIRST CONTRIBUTION TO THE STUDY OF WHEAT BUNT IN ITALY *

In a paper published in 1942, Prof. T. SĂVULESCU reported his original discoveries regarding bunt of wheat in Rumania. They threw fresh light on the knowledge of this disease and on new control methods. As this author says, this paper is only the first publication of numerous investigations started over ten years ago by himself and his many collaborators with a view to ascertaining exactly the biology of wheat bunt in Rumania, the species which exist, the wheat varieties most attacked and all other information which might contribute towards solving such an important problem.

In Italy, identical studies were begun a short time ago at the Institute of Plant Pathology in Florence under the impetus of the Director, Prof. C. SIBILIA, who entrusted me with the investigations about which I will speak here. For the moment I will only note the results of the preliminary observations and the points which will be thoroughly examined in subsequent communications.

Because of the war during which this investigation was begun, I was unable to procure abundant material for observation. What I did succeed in obtaining, through Prof. SIBILIA himself, came from Central and South Italy. I was only able to have caryopses from Bari, Gravina in Puglia, Bari Province, Anagni, Frosinone Province, Campi Bisenzio and Pelago, Florence Province. Owing to difficulties in contacting the other regions of the country, no research was started for North Italy. Shortly the plan of investigation will be enlarged and will include the collection of samples from many other regions in order to be able to have a general and complete table, all the more so as during this period of war, because of the shortage of copper fungicides used against bunt, there was an appreciable increase in infection.

Geographical distribution of wheat bunt in Italy. — In treatises on plant pathology and in works on allied subjects it is stated that bunt is very frequent in Italy. At the moment it is represented by the two species *Tilletia levis* (Kühn) [*T. foetens* (Berk. et Curt.) Trel.] and *T. tritici* (Bjerk.) Wint. The first species is widespread in Central and South Italy and the second in North Italy.

From the examination of my samples which all, I repeat, came from Central and South Italy, I found that the greater part of attack (about 98 per cent.) was due to *T. levis* with brown smooth chlamydoconidia, 16.2-17.9 μ in diameter, while hardly 2 per cent. was represented by *T. tritici* with brown echinulated chlamydoconidia, 17.1-18.2 μ in diameter. These percentages

* Communication from Dr. VINCENZO GRASSO, Assistant at the Institute of Plant Pathology of the University of Florence, transmitted by Prof. CESARE SIBILIA, Director of this Institute.

are the same for all the localities mentioned above, and for the infected wheat varieties: 'Mentana', 'Roma' and 'Frassineto'.

T. levis is characterized by great variety in the shape of the chlamydoconidia: spherical, elongated, oval, tetrahedral, curved; echinulated *T. tritici* generally has a spherical shape. The height of the crests of the chlamydoconidia of this latter species vary considerably: from the maximum dimension of 1.7-1.9 μ it passes to lower values for which it would be interesting to ascertain if there are other echinulated species in Italy, as G. GASSNER and T. SAVULESCU proved respectively for *T. intermedia* in Turkey and *T. triticoides* in Rumania.

Coexistence of the two species (*T. levis* and *T. tritici*). — To date the existence of the two species of *Tilletia* in Italy was known as occurring separately on different caryopses. From my recent investigations I found that these two species can exist in nature at the same time on the caryopsis, irrespective of the locality and the variety of wheat attacked *.

The numerical ratio between the chlamydoconidia of the two co-existent species is very small, practically 1 : 1000; namely, that *T. levis* is very abundant while *T. tritici* is scarce.

The biometric measurements of the chlamydoconidia of these fungi living in the same caryopsis, are the same as those of the species living on separate caryopses.

Partial infection of the caryopses. — An entirely new fact in the study of wheat bunt in Italy acquires a special signification from this point of view; it is a question of the presence of partially bunted grain.

On examining the different samples I observed that in one inflorescence, besides completely infected caryopses, there were also others which showed partial infection as can be seen in figures 1 and 2 which show the grain back (fig. 1) and front (fig. 2). In the first 5 caryopses infection began at

Figure 1.

Figure 2.

* This observation is particularly interesting as it corresponds to the result obtained artificially by AJROLDI.

the embryo and subsequently spread to other zones of the grain up to total infection. In the last two caryopses, infection began at different points away from the embryo.

From the preliminary observations it results that the mass of chlamydoconidia is formed by *T. levis* and is developed between the pericarp and the tegument of the endosperm.

The fact of having found caryopses partially infected is not only of theoretical importance, but, on the contrary, of especial practical value since it calls for fresh directives in the method of control to be employed against this disease. If so far it has been held that bunt should be controlled by using dusts on the caryopses, since it was thought that the chlamydoconidia adhere only to these, now that in grain to a large extent healthy, partial infection has also been found where the chlamydoconidia are in the inside, another method will have to be adopted to reach not only the germs of the fungus on the outside of the grain, but also those inside. It is not our desire for the moment to change the method of control, moreover, partially infected grain is not very frequent, though, on the other hand, steps should be taken to prevent a further spread of the disease, and consequently we advise against using for seed, batches of wheat attacked by bunt.

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THE COLORADO BEETLE IN FRENCH SWITZERLAND *

Situation on May 1, 1946.

The conditions for hibernation of the Colorado beetle (*Leptinotarsa decemlineata*) during the 1945-46 winter were extremely favourable. A very early spring caused an early appearance of the beetle, even before the newly planted potatoes had sprouted. As early as March 14, it was reported in Geneva that full grown beetles brought to the surface by mining operations were moving about the ground. At the end of March, numerous emergences were noted in the basin of the Leman and the Broye Plain.

From the end of April, the parasite was reported in large numbers in the Geneva Canton on early potatoes and to a large extent in the Friburg communes of the Grand Marais. Some emergences were even observed in high-lying regions as at Rossinière.

The first oviposition has already begun and in general a heavy invasion of the parasite is expected. In the Jura region, however, mainly in the Neuchâtel Canton, the persistent drought appears to have temporarily checked the emergence of full-grown beetles.

This early invasion of insects having hibernated on the spot will soon be augmented by swarms from seriously infected regions.

Situation on June 1, 1946.

During May the Colorado beetle situation in French Switzerland developed slowly. In severely invaded regions, where hibernation conditions for the parasite are favourable, there was a very heavy emergence of perfect insects and large numbers are to be found on potato plants. Hatching was incomplete and there seems to have been some abortion. This slowing down in invasion is to be attributed to the relatively low night temperatures during May. At the end of May young larvae were found in low-lying regions.

In high-lying regions, the insects are not very numerous and hand collection is sufficient to check the spread of the parasite. The allocation of prizes to school children for collecting the insects, a practice followed in certain cantons and in many communes encourages conscientious work to the benefit of agriculture.

The application of treatments was rightly deferred until the appearance of medium sized larvae and until recent eggs were hatched.

While last year, already in May, numerous swarms of Colorado beetles appeared in widely differing parts of the country, this year at the end of

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* Communication from the Federal Experiment and Seed Testing Institution, Lausanne, Mont Calme, Switzerland.

May, the insect had not yet started to fly. At the most, on hot days, only a few beetles were to be seen trying to fly.

In brief, as a result of the weather conditions in May, the spread of the Colorado beetle in French Switzerland was checked. The parasite is numerous in the low-lying regions, but the situation remains latent and insecticidal treatments will only be applied when the larvae have definitely appeared.

Situation on July 1, 1946.

The weather in June, with the temperature below the average, heavy rainfall (Champs de l'Air 198.6 mm. compared with the average 99 mm. and 51 mm in 1945), the absence of strong winds, was not conducive to the spread of the Colorado beetle in French Switzerland. On the only really hot days of the month, in particular June 30 (30.6° C.), there was no wind thus preventing the formation of swarms carried to a distance. The invasion remained so to speak, localized without the addition of insects from distant regions. Under these conditions a true idea can be obtained of the extent of the Colorado beetle inside the country. Regions appearing free from the insect are in reality more or less infected. The cause probably lies in the exceptional winter conditions of 1945-46. Thus, for example, the advance of the parasite in the fore-Alps is evident.

The wide variations in temperature during June made hatching very irregular. At present larvae are found in all stages of development. Deciding the right moment for treatments is particularly difficult. Preference will be given to the use of calcium arsenate for treating crops infested by young larvae which will be easily killed by absorbing the poison when feeding on the leaves, while DDT, Toxaline or Derris dusts will be employed to destroy the sluggish larvae in an advanced stage of development for which the use of arsenates will only provoke premature nymphosis.

The particularly wet weather in June 1946, the difficulty the farmers had in haymaking rendered the application of treatments somewhat arduous. The situation is dangerous as the parasite has had the time in many cases, to attain full development and to assure, if the weather is propitious, an abundant summer generation. Although the damage caused this year by the Colorado beetle appears less than in the previous year, the fact still remains that the situation is serious and probably numerous regions will go through a period of permanent implantation of the parasite.

LEGISLATIVE AND ADMINISTRATIVE MEASURES

Belgium. — By Decree of the Regent dated March 11, 1946 all elms showing evident signs of attack by the so called Dutch elm disease (*Graphium ulmi*), wherever located, must be reported, indicating the circumference at 1.50 m. from the ground, by the proprietor or his agent to the forest inspector of the province before July 1 of each year, and for the first time in the same month in which this Decree is published in the *Moniteur belge*. These trees will be felled at the time and within the period established by the aforesaid authority.

In default of the proprietor felling the trees within the period ordered, felling with be carried out officially and at the expense of the owner, without prejudice to penalities.

This Decree substitutes that of August 21, 1942 [see this *Bulletin* 1942, No. 11, p. 158]. (*Moniteur belge*, 23 mars 1946, 116e année, nº 82, p. 2662-2663).

*** By Decree of the Regent of the same date, all poplars showing evident signs of attack by 'poplar canker', wherever located, must be reported, indicating the circumference of the tree at 1.50 m. from the ground, by the proprietor or his agent, to the forest inspector of the district before July 1 of each year, and for the first time during the month in which this Decree is published in the *Moniteur belge*. The trees will be felled at the time and within the period decided by this authority.

If the proprietor does not fell the trees within the period ordered, felling will be carried out officially and at his expense without prejudice to penalties. (*Ibid.*, 24 mars 1946, nº 83, p. 2690-2691).

*** By Decree of the Regent dated May 6, 1946, every proprietor, lessee, tenant or occupant, of whatever status, who finds on the land he occupies, whatever the crop grown, the presence of the Colorado beetle [*Leptinotarsa decemlineata*] in the form of the perfect insect, egg, larva or nymph, is required to destroy same immediately.

The burgomaster sees to the strict enforcement of this measure.

In the event of the parties concerned failing to use effective means of destruction or when these means prove inadequate, the burgomaster has to provide officially for the destruction of the Colorado beetle on the territory of his commune.

This duty also devolves upon the governor, either for the entire province or for that part where these measures are deemed necessary.

Preventive and curative treatments can be ordered by the Minister of Agriculture or by his deputy as well as by the provincial governors, at the time and place which they may determine.

The use of certain products or certain methods of treatment may be prohibited, brought under regulation or prescribed, either through ministerial instructions or circulars, or through notes, instructions or circulars issued by the special Phytopathological Service.

In the regions where horticultural plants are cultivated for export, all potato crops located within a radius of 10 kilometres from horticultural establishments must be adequately treated with calcium or lead arsenate at least twice a year, or else with contact poisons, such as rotenone, at least three times a year.

The provincial governors are required to see that this provision is put into effect.

Unless allowed by the special Phytopathological Service, the cultivation of potatoes, tomatoes and eggplants is prohibited in horticultural enterprises which grow plants for export.

This rule can also be extended to other plants.

The inspection certificate is denied to or withdrawn from the grower who does not conform to this regulation.

The transportation or keeping, in any manner whatsoever, of live Colorado beetles is at all times prohibited.

This regulation may be waived, chiefly for scientific purposes.

The agents commissioned with supervising the enforcement of this Decree are authorized to inspect, between sunrise and sunset, any cultivated land where they consider their presence necessary in regard to Colorado beetle control.

Infringements of the provisions of this Decree will be punished in conformity with the provisions of Articles 5, 6 and 7 of the Law of December 30, 1882 on the sanitary control of domestic animals.

Without prejudice to the rights incumbent upon police officers and the authority of their reports, inspectors, assistant inspectors and technical officials and assistants of the special Phytopathological Service, State agronomists and horticultural advisers are invested with the right to look for, in any part of the country, and to ascertain, by reports authentic unless otherwise proved, the infringements of the provisions of this Decree.

Notwithstanding Article 3 of the Royal Decree dated March 26, 1936 [see this *Bulletin* 1936, No. 7, p. 155], the presence of the Colorado beetle does not have to be reported to the burgomaster of the commune nor to the Minister of Agriculture. (*Moniteur belge*, Bruxelles, 18 mai 1946, 116e année, nº 138, p. 4343-4344).

*** By Ministerial Decree of May 7, 1946, the Ministerial Decree of March 28, 1945 [see this *Bulletin* 1945, Nos. 9-10, pp. 79-80] relative to measures for preventing the spread of the Colorado beetle is repealed. (*Ibid.*, p. 4345).

Italy. — Ministerial Circular No. 109 of December 17, 1945 deals with the organization of the control of the Colorado beetle [*Leptinotarsa decem-*

lineata] in the country. (*Bollettino Ufficiale del Ministero dell'Agricoltura e delle Foreste*, Roma, dicembre 1945, anno XVII, n. 12, pp. 2388-2389).

*** Legislative Decree of the Lieutenancy No. 244 of February 22, 1946 indicates the terms and conditions to be observed for the payment of the expenses incurred for locust control operations. (*Gazzetta Ufficiale del Regno d'Italia*, Roma, 9 maggio 1946, anno 87°, n. 106, p. 982).

*** Ministerial Decree dated March 20, 1946 lists the wild rabbit among the animal pests in the territory of the commune of Nettuno. (*Ibid.* Roma, 27 marzo 1946, n. 72, p. 634).

*** By Ministerial Decree of April 9, 1946 an Advisory Commission has been set up to assist the General Commissariat for the control of citrus scales in Sicily and Calabria, with headquarters at Catania. (*Ibid.*, 6 maggio 1946, n. 104, p. 961).

*** By Legislative Decree of the Lieutenancy No. 222 of April 19, 1946, the expenditure authorized by Legislative Decree of the Lieutenancy No. 806, dated November 23, 1945 [see this *Bulletin* 1946, Nos. 3-4, p. 30] to cover the cost of locust control operations has been increased by 150,000,000 lire. (*Ibid.*, Roma, 3 maggio 1946, n. 102, p. 933).

French Morocco. — By Decree of the Director of Economic Affairs, dated November 20, 1945, the Decree of September 10, 1936 [see this *Bulletin*, 1936, No. 11, p. 247], relative to the sanitary policy governing the importation of vegetables is supplemented by an Article 2 *ter* drawn up as follows:

'Art. 2 *ter*. — Consignments of potatoes, tomatoes or egg-plants should not contain any parasite and in particular should not be attacked by the Colorado beetle (*Leptinotarsa decemlineata* Say) potato wart disease (*Synchytrium endobioticum* Schilb.) and the following virus diseases: potato spindle tuber, potato unmottled curly dwarf, potato witches' broom, Canada streak, potato calico; there should be no earth, tops or other debris with the produce. These consignments require to be inspected before dispatch by an officer of the Plant Protection Service of the country of origin and accompanied by a certificate conformable to the example attached to this Decree. The bags or containers must be sealed or ticketed under the supervision of the aforesaid officer'. (*La Terre Marocaine*, Casablanca, février 1946, 16e année, n° 195, p. 25).

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